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Stefan Szczepan Wroclaw University of Health and Sport Sciences, Poland, Stefan.Szczepan@awf.wroc.pl

Kamil Michalik Wroclaw University of Health and Sport Sciences, Poland, kamil.michalik@awf.wroc.pl

Rafał Hebisz Wroclaw University of Health and Sport Sciences, Poland, rafal.hebisz@awf.wroc.pl

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Abstract

Background: The aim of study was to investigate if differences appeared in haematological parameters after a 6-week moderate-intensity swimming intervention with added respiratory dead space volume (ARDSV) in recreational swimmers.

Material and methods: A sample of 22 individuals were divided into an experimental (E) and a control (C) group, tested for maximal oxygen uptake (VO₂max). The intervention involved 50 min. of front crawl swimming performed at 60%VO₂max twice weekly for 6 weeks. ARDSV was induced via tube breathing (1000ml) in group E during each intervention session. Haematological parameters measured before and after the intervention included red blood cell concentration (RBC), hemoglobin concentration (HGB), haematocrit (HCT), mean cell haemoglobin mass (MCH), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), white blood cells (WBC), lymphocytes (LYM), monocytes (MON), and granulocytes (GRA).

Results: The pre- and post-analysis revealed significant (p< 0.05) changes for groups E and C in the following variables: MCH (E: increase by 4.10%; C: inc. by 3.28%), MCV (E: inc. by 9.39%; C: inc. by 7.48%), and MCHC (E: decrease by 3.52%; C: dec. by 4.05%).

Conclusions: Adding ARDSV to routine moderate-intensity swim training does not improve physical capacity or stimulate adaptation in haematological parameters among physically active individuals.

Keywords

swimming, added respiratory dead space volume (ARDSV), maximal oxygen uptake (VO2max), haematological parameters

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Cover Page Footnote

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Article

Does a six-week intervention with added respiratory dead space volume in swimming improve haematological and immunological status?

Stefan SZCZEPAN1*, Kamil MICHALIK2, Rafał HEBISZ3

- ¹ Department of Swimming, Faculty of Physical Education and Sport Science, Wroclaw University of Health and Sport Sciences, Poland, ORCID 0000-0002-5075-7357
- ² Department of Human Motor Skills, Faculty of Physical Education and Sport, Wroclaw University of Health and Sport Sciences, Poland, ORCID 0000-0002-1296-0434
- ³ Department of Physiology and Biochemistry, Faculty of Physical Education and Sports, Wroclaw University of Health and Sport Sciences, Poland, ORCID 0000-0002-8474-6461
- * Correspondence: Stefan Szczepan, Ph.D., Department of Swimming, Faculty of Physical Education and Sport Science, Wroclaw University of Health and Sport Sciences, Poland; Ignacego Jana Paderewskiego 35, Swimming pool, 51-612 Wroclaw, Poland; Phone: 0048 71 347 3404, Fax: 0048 71 347 3450, e-mail: stefan.szczepan@awf.wroc.pl

Abstract: Introduction: The aim of study was to investigate if differences appeared in haematological parameters after a 6-week moderate-intensity swimming intervention with added respiratory dead space volume (ARDSV) in recreational swimmers. Material and Methods: A sample of 22 individuals were divided into an experimental (E) and a control (C) group, tested for maximal oxygen uptake (VO2max). The intervention involved 50 min. of front crawl swimming performed at 60%VO2max twice weekly for 6 weeks. ARDSV was induced via tube breathing (1000 ml) in group E during each intervention session. Haematological parameters measured before and after the intervention included red blood cell concentration (RBC), hemoglobin concentration (HGB), haematocrit (HCT), mean cell haemoglobin mass (MCH), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), white blood cells (WBC), lymphocytes (LYM), monocytes (MON), and granulocytes (GRA). Results: The pre- and post-analysis revealed significant (p < 0.05) changes for groups E and C in the following variables: MCH (E: increase by 4.10%; C: inc. by 3.28%), MCV (E: inc. by 9.39%; C: inc. by 7.48%), and MCHC (E: decrease by 3.52%; C: dec. by 4.05%). Conclusions: Adding ARDSV to routine moderate-intensity swim training does not improve physical capacity or stimulate adaptation in haematological parameters among physically active individuals.

Keywords: swimming, added respiratory dead space volume (ARDSV), maximal oxygen uptake (VO2max), haematological parameters.

1. Introduction

Maximal oxygen uptake (VO₂max) represents the upper limit of energy supply for aerobic processes and is a key determinant of endurance capacity [1]. It constitutes a strong and independent predictor of overall and disease-related mortality [2]. The VO₂max value, being a complex feature of aerobic capacity, expresses the efficiency of the respiratory, circulatory, and muscular systems in transporting and utilizing oxygen in energy processes [3].

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Copyright: © 2022 by Gdansk University of Physical Education and Sport. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC-BY-NC-ND) licenses (https://creativecommons.org/licenses/ by/4.0/). Numerous physiological factors interact in a complex way to regulate this variable. As illustrated by Fick's equation, one can broadly divide them into 'central' and 'peripheral' determinants, highlighting cardiac output and arteriovenous blood oxygen content difference. Blood volume and haemoglobin concentration (HGB) play an equally important role in respiratory gas transport [4]. In addition, haemoglobin is involved in buffering blood pH changes by transporting CO₂ and by binding to H⁺ ions [5]. It is also accepted that a VO₂max increase induced by regular training is largely related to a rise in red blood cell volume [1, 6]. Warburton et al. [7] reported that post-workout (12-week) hypervolaemia accounted for ca. 47% of the VO₂max change. Therefore, ways are sought

population. One of the methods to positively affect the cardiopulmonary system function and metabolic processes involves inducing a state of increased partial pressure of carbon dioxide in the blood (above 45 mm Hg), which is referred to as hypercapnia [8]. A simple way to induce hypercapnia is to apply an added respiratory dead space volume (ARDSV). This is achieved by breathing through a tube of a specific volume [9, 10, 11]. In proportion to ARDSV, some amount of the inhaled air is mixed with the exhaled air, which, additionally, is heated. The impediment of CO₂ removal from the lungs results in a change of the breathing air composition, increased CO₂ content, and maintenance or slight decrease in oxygen content [12]. As a consequence, the pressure gradient of CO₂ between the alveoli and the blood decreases, and the slowing of the diffusion rate in the lungs provokes an increase in its blood pressure [12, 13].

to trigger adaptive changes that will bring effective and measurable health benefits to the

Several experiments have confirmed the effectiveness of using ARDSV of 1000 ml in the development of physical capacity (expressed as an increase in VO₂max) in regular continuous training of road cyclists [9], as well as in high-intensity interval training of swimmers [14] and triathletes [15]. In comparison, a 30–50-minute training at an intensity level of 60% VO₂max performed for 6 weeks (twice a week) with the similar ARDSV volume did not improve VO₂max in young physically active men [16] or in recreational swimmers [10]. Thus, further or additional research is needed to better understand the influence of ARDSV on physical performance or health status.

Few studies describe the effect of regular training with ARDSV on the concentrations and sizes of blood morphotic elements. Zatoń et al. [17] reported a statistically significant decrease in HGB, red blood cells (RBC), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) as a result of a 5-week experiment. Off-road cyclists performed moderate-intensity 1-hour efforts 3 times a week (between the aerobic and anaerobic threshold) while breathing through 1000 ml ARDSV. The authors pointed to an increase in the plasma volume (related to the thermoregulatory function of blood) and a rejuvenation of the red blood cell population as a possible mechanism of the induced changes. This leads to a reduction in blood viscosity and in the friction of blood cells against blood vessel walls [17]. At the same time, no change in VO2max was indicated, so further research is needed to investigate changes in the number and concentration of blood morphotic elements which may indicate an improvement in gas transport and increased VO2max as a result of regular training with ARDSV.

In swimming, as an endurance discipline, oxygen transport is the fundamental condition for achieving high sports results among professional swimmers [18] and maintaining health in recreational swimmers [19]. Although the share of the aerobic energy production system depends on the swimming distance and swimming style specialization [20], the swimming training of professionals and amateurs should emphasize the development of aerobic capacity, which is determined by effective oxygen transport [21].

It is also well known that blood volume and haemoglobin concentration play an important role in respiratory gas transport and aerobic capacity, including achieving maximum oxygen uptake (VO₂max) [22]. It is thought that swimmers with a higher VO₂max may endure a large volume of training, swim faster, and maintain the economy of movement in the water and recover sooner after physical effort [23]. The relationship between

VO₂max and swimming speed is also known [24]. For recreational swimmers, VO₂ is a health predictor [2].

Additionally, haemoglobin is involved in buffering changes in blood pH by transporting CO₂ and binding to H⁺ ions [25]. Effective buffering mechanisms ensure the neutralization of high concentrations of hydrogen ions and prevent a reduction in the ability to maintain a high intensity of exercise [26].

Furthermore, endurance athletes, including swimmers, have been diagnosed with haematological abnormalities, i.e. decreased haemoglobin concentration levels [27]. A decrease in the number of erythrocytes and their mean cell volume is also a frequent reaction to intensive workout in water [28]. In this context, the search for methods to enhance the concentration of blood variables seems appropriate.

The aim of this study was to examine whether there appeared any differences in haematological parameters after a 6-week moderate-intensity swimming intervention with ARDSV in recreational swimmers. It was hypothesized that the ARDSV intervention would bring about large improvements in haematological parameters with an accompanying increase in VO₂max values. To our knowledge, this theory has not been empirically addressed yet. The rationale behind these postulates comes from the research on ARDSV which has shown positive effects on selected haematological variables.

2. Materials and Methods

2.1. Participants

The research involved 22 healthy and physically active individuals, 11 males and 11 females. The participants were former swimmers with an average competitive experience of 6 ± 2 years. Three years passed from the end of their swimming career to the time of the experiment. During this time their physical activity was limited to swimming an average distance of 2 km twice a week with an intensity of 65–75% of the maximal heart rate (HRmax), assessed on the basis of individual fitness tests. Hence the research group was classified as recreational swimmers.

The following inclusion criteria were applied: age (adults, age range 20–30 years), homogeneous in terms of somatic build (body height, body mass), front crawl swimming ability, and no contraindications against effort.

The participants were divided into 2 groups: the control (C) group (7 men, 4 women; age: 24.00 ± 3.35 years; body height: 168.06 ± 27.34 cm; body mass: 72.32 ± 10.13 kg; VO₂max: 47.09 ± 8.85 ml · kg⁻¹ · min⁻¹) and the experimental (E) group (4 men, 7 women; age: 24.27 ± 2.69 years; body height: 173.36 ± 9.08 cm; body mass: 70.03 ± 13.14 kg; VO₂max: 45.55 ± 7.47 ml · kg⁻¹ · min⁻¹). The groups were compared in terms of the somatic parameters, i.e., age (p = 0.72), body height (p = 0.50), body mass (p = 0.65), and VO₂max (p = 0.65). The Wilcoxon nonparametric test was applied in the assessment (alpha error: 0.05). This generated comparable groups with an objective baseline level of somatic build.

Before entering the experiment, all swimmers provided their written consent to participate in the study; they could withdraw at any time. The experiment was approved by the University Research Ethics Committee (#14/2017) and carried out in accordance with the standards of the Declaration of Helsinki.

2.2. Design and Procedures

A week before the start of the research protocol, a familiarization session was held to adapt the participants to the study protocol with ARDSV, as none of them had previously used this device. The familiarization session involved a 1000 ml low-intensity front crawl swimming in a 25-m indoor swimming pool, with breathing through an ARDSV device. In the subsequent week, resting haematological measurements and an incremental VO₂max exercise test on a cycle ergometer were performed. The training comprised a 6week intervention (twice per week). One week after the end of training, the same tests were performed as at the beginning of the experiment.

2.3. Measurements of Haematological Parameters

Haematological parameters were tested 3 days before and after the ARDSV intervention to assess changes. The testing series were performed in the same controlled conditions (temperature: 24°C, relative humidity: 50%) in a climate-controlled exercise laboratory (PN-EN ISO 9001:2009 certified). The measurements were taken by a trained laboratory technician.

The haematological assessments in capillary blood collected from a fingertip before the incremental test were performed with an ABX Micros OT 16 analyser (Horiba Medical, Kyoto, Japan). Red blood cell concentration (RBC) [$10^6 \cdot (mm^3)^{-1}$], haemoglobin concentration (HGB) [$g \cdot dl^{-1}$], haematocrit (HCT) [%], mean cell haemoglobin mass (MCH) [pg], mean cell volume (MCV) [μm^3], mean cell haemoglobin concentration (MCHC) [$g \cdot dl^{-1}$], white blood cells (WBC) [$10^3 \cdot (mm^3)^{-1}$], lymphocytes (LYM) [$10^3 \cdot (mm^3)^{-1}$], monocytes (MON) [$10^3 \cdot (mm^3)^{-1}$], and granulocytes (GRA) [$10^3 \cdot (mm^3)^{-1}$] were determined following the protocol by Zatoń et al. [17].

2.4. Incremental Exercise Test

All participants' body mass [kg] and height [cm] were measured by using WPT 200 medical scales (Radwag, Radom, Poland). The incremental exercise test on a cycle ergometer was applied to assess VO₂max [ml \cdot kg⁻¹ \cdot min⁻¹] and performed 3 days prior the ARDSV intervention in accordance with the protocol by Michalik et al. [29]; an Excalibur Sport cycle ergometer (Lode BV, Groningen, the Netherlands) was used. HR [beats \cdot min⁻¹] was continuously measured with a non-invasive HR monitor (S810, Polar Electro, Kempele, Finland). Gas exchange was evaluated breath-by-breath by using a metabolic cart (Quark b2, Cosmed, Rome, Italy). The device was calibrated with a reference gas mixture of CO₂ (5%), O₂ (16%), and N₂ (79%). Pulmonary function assessment began 2 minutes prior to the test start and continued 5 minutes after the test conclusion, with data averaged over 30-second intervals. VO₂max was automatically calculated. It was defined as the highest 30-second average at which relative VO₂ values plateaued (<1.35 ml \cdot kg⁻¹ \cdot min⁻¹) despite an increase in workload or 2 of the following criteria: (a) respiratory exchange ratio > 1.10; (b) attainment of HRmax (within 10 beats \cdot min⁻¹ of age-predicted maximum [220-age]); (c) voluntary exhaustion [30].

2.5. Training

The individuals' assignment to the study groups was based on VO₂max values measured during the incremental exercise test. The VO₂max values were arranged from the highest to the lowest. The study participants were ascribed sequential numbers in accordance with their VO₂max results. The individuals with odd numbers were assigned to group E and those with even numbers to group C.

The participants in group E swam twice a week for 6-weeks utilizing the ARDSV training, for a total of 12 swimming sessions. During each 50-minute session, all swimmers used only the front crawl technique. The total distance in meters was not measured. The interval between sessions was 72 hours. During each swimming session, the subjects undertook constant, moderate-intensity physical effort (the aerobic pathway). The effort intensity was individually determined on the basis of the HR achieved at 60% VO₂max in the incremental exercise test, corresponding to individual HR values in the range of 125–140 beats · min⁻¹. While swimming, the participants monitored their HR with an RS400 sports watch (Polar Electro, Kempele, Finland). Intensity below the lactate threshold was chosen because it was suitable for long-term effort of individuals involved in the experiment.

Group E swam with a custom ARDSV apparatus consisting of a polypropylene centremount swimming snorkel with a mouthpiece (Speedo International Ltd., Nottingham, UK) integrated with 2.5-cm diameter ribbed tubing to provide ARDSV of 1000 ml (Figure 1). Dead space volume (1000 ml) was identical for each participant and measured by filling the snorkel with water and then transferring the volume to a graduated cylinder, as described by Szczepan et al. [11]. The snorkel was sufficiently rigid to maintain a constant volume when swimming. The swimmers in group C took part in the same training but without the ARDSV intervention. In group C, no additional respiratory changes were introduced; the group applied a standard breathing pattern for the front crawl technique.

Based on the previous studies, a state of increased partial pressure of carbon dioxide in the blood (above 45 mmHg) was found, while the integrated with 2.5-cm diameter ribbed tubing to provide ARDSV of 1000–1200 ml has been used. In the laboratory conditions, the end-tidal partial pressure of carbon dioxide PETCO₂ during cycloergometer test at the intensity of 60% VO₂max with ARDSV of 1000 ml was 47 (mmHg) [31]. Furthermore, carbon dioxide partial pressure pCO₂ in blood during the swimming at an intensity of 60% VO₂max with ARDSV of 1200 ml was 49 (mmHg) (unpublished data).

All sessions took place in a 25-metre swimming pool, under uniform conditions (water temperature: 27°C, air temperature: 28°C, relative humidity: 60%, lighting: 600 lx). Throughout the study, the individuals from both groups led a lifestyle and maintained a diet normal for people of that age and did not participate in any additional training. The participants' diet was not controlled.



Fig 1. A custom added respiratory dead space volume (ARDSV) apparatus consisting of a polypropylene centre-mount swimming snorkel with a mouthpiece (Speedo International Ltd., Nottingham, UK) integrated with 2.5-cm diameter ribbed tubing to provide 1000 ml of dead space.

2.6. Statistical Analysis

The quantitative investigation planning involved a 4-dimensional approach (alpha, power, sample size, and effect size) and followed the accepted methodology [32]. Data are presented as means \pm standard deviations, the difference (Δ) between pre- and post-intervention values, and the standard deviation for the difference. Furthermore, 95% confidence intervals are given. Parameter changes (increase or decrease) are expressed as a dimensionless ratio of two quantities [%]. Significance was set at an alpha level of 0.05 for all statistical procedures, with p values provided for all results. The distribution of the data set was screened for normality by using the Shapiro-Wilk test, and the homogeneity of variances was checked with Levene's test. Haematologic outcomes (RBC, HGB, HCT, MCH, MCV, MCHC, WBC, LYM, MON, and GRA) were compared with the use of two-factor repeated measures ANOVA (group × time) for dependent samples and Scheffé's test for pairwise post-hoc comparisons. Effect sizes were calculated by using partial eta

squared (η_p^2) . The effect sizes were interpreted as small (0.02), moderate (0.13), or large (≥ 0.26) [33, 34]. All calculations of the analysed variables were performed with the Statistica version 13.1 software package (StatSoft, Tulsa, USA) and the IBM SPSS Statistics version 26 software package (IBM Inc., Chicago, USA). The sample size was estimated with a stand-alone power analysis program for statistical tests (G*Power 3.1.9.2, Kiel University, Germany) [35] with a small effect size of $f^2 = 0.29$. With the assumption of an alpha error of 0.05 and power of 0.80, the required total sample size was estimated to be 26 subjects. Due to the length and commitment of the intervention, it was possible to include only 22 individuals in the final analysis.

3. Results

The pre- and post-intervention haematological parameters for repeated measurement comparisons are presented in Table 1. The difference analysis revealed changes for groups C and E in the MCH variable (E: increase by 4.10%, p = 0.001; C: increase by 3.28%, p = 0.001; $\eta_p^2 = 0.84$). For the MCV variable, the difference analysis also showed changes for both groups (E: increase by 9.39%, p = 0.04; C: increase by 7.48%, p = 0.001; $\eta_p^2 = 0.87$). Similarly, for both groups (C and E), the difference analysis determined changes in the MCHC variable (E: decrease by 3.52%, p = 0.001; C: decrease by 4.05%, p = 0.001; $\eta_p^2 = 0.82$). Among haematological (immunological) variables (Table 2), pre- and post-intervention comparisons did not indicate any other changes. VO₂max presented no significant changes between the pre- and post-intervention status.



Tuble 1. Changes in selected factuation parameters (NDC, 110D, 1101, 1	nges in selected haematological parameters (RBC, HGB, HCT, MCH, MCV, MCHC) and VO2max after the	e ARDSV interventior
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	Norms		Before intervention			After intervention			Post- to pre-intervention change						
Variable		Group	Mean	SD	(95% CI)	Mean	SD	(95% CI)	Δ (post-pre)	SD of Δ (post-pre)	% difference	F (ANOVA)	p-value (post-hoc)	η_p^2	
RBC	2050	Е	4.76	0.39	4.50-5.02	4.57	0.42	4.29-4.85	-0.19	0.57	-3.92	7.05	0.19	0.00	
10 ⁶ ·(mm ³) ⁻¹	3.8-5.8	С	4.82	0.41	4.54-5.10	4.69	0.49	4.36-5.02	-0.14	0.64	-2.81	7.85	0.45	0.28	
HGB	11.0-16.5	11.0.17 5	Е	13.71	1.32	12.82-14.59	13.71	1.33	12.81-14.60	0.00	1.87	0.00	0.00	1.00	0.00
g·dl⁻¹		С	13.78	1.78	12.58-14.98	13.83	1.92	12.54–15.12	0.05	2.62	0.33	0.02	0.99	0.00	
НСТ	35.0-50.0		Е	40.21	3.42	37.91-42.51	41.70	3.78	39.16-44.24	1.49	5.10	3.71	11 (0	0.24	0.07
[%]		С	40.41	4.55	37.35-43.46	42.27	5.45	38.61-45.93	1.86	7.10	4.61	11.69	0.10	0.37	
MCH			Е	28.83	0.96	28.18-29.47	30.01	1.05	29.31-30.71	1.18	1.42	4.10	100 (5	0.00*	0.04
[pg]	26.5-33.5	С	28.57	2.28	27.04-30.10	29.51	2.49	27.84-31.18	0.94	3.37	103.65 3.28	0.00*	0.84		
MCV	~~~~~~	Е	83.27	5.82	79.37-87.18	91.09	2.77	89.23–92.95	7.82	6.44	9.39		0.04*	a a -	
[µm³]	80.0-97.0	С	83.82	5.74	79.96–87.68	90.09	6.58	85.67–94.51	6.27	8.73	7.48	128.61	0.00*	0.87	
MCHC	31.5-35.0	Е	34.10	0.50	33.77–34.43	32.90	0.62	32.48-33.32	-1.20	0.79	-3.52	00.01	0.00*		
g·dl ⁻¹		С	34.08	0.80	33.54-34.62	32.70	0.54	32.33-33.07	-1.38	0.97	-4.05	93.81	0.00*	0.82	
VO2max	-	E	45.55	7.47	40.53-50.58	46.70	8.36	41.11-52.30	1.15	11.21	2.52		0.85		
[ml·kg ⁻¹ ·min ⁻¹]		С	47.10	8.85	41.15-53.04	47.60	10.17	40.76-54.43	0.50	13.48	1.06	0.83	0.98	0.04	

Data presented as mean \pm standard deviation and confidence intervals; *significant difference at p < 0.05 vs. the pre-intervention value; Δ and % difference with respect to the pre-intervention status; positive Δ indicates an increase in variables; positive % indicates an increase in variables; η_p^2 – partial eta squared for repeated measurements; F – Fischer's F statistics for repeated measurements; p-value (post-hoc) – Scheffé's test for pairwise post-hoc comparisons; E – experimental group, C – control group; RBC – red blood cells, HGB – haemoglobin concentration, HCT – haematocrit, MCH – mean cell haemoglobin, MCV – mean cell volume, MCHC – mean cell haemoglobin concentration, VO₂max – maximal oxygen uptake; the VO₂max variable values derived from Szczepan et al. [11]

		ms Group	Ве	efore inte	rvention	After intervention			Post- to pre-intervention change									
Variable	Norms		Mean	SD	(95% CI)	Mean	SD	(95% CI)	∆ (post-pre)	SD of ∆ (post-pre)	% difference	F (ANOVA)	p-value (post-hoc)	η_p^2				
WBC	3.5-10.0		E	7.97	4.11	5.21-10.73	6.60	1.56	5.55–7.65	-1.37	4.39	-17.22	0.05	0.34	0.00			
10 ³ ·(mm ³) ⁻¹		С	5.55	1.14	4.77-6.31	5.11	0.92	4.49-5.73	-0.44	1.47	-7.87	3.07	0.95	0.09				
LYM	1.2-3.2	1000	1000	1000	1000	Е	2.11	0.60	1.70-2.51	2.37	0.52	2.02-2.72	0.26	0.79	12.32	0.001	0.51	0.00
10 ³ ·(mm ³) ⁻¹		С	2.19	0.66	1.75-2.61	1.92	0.50	1.58-2.26	-0.27	0.83	-12.33	0.001	0.48	0.00				
MON	0.3-0.8	E	0.35	0.13	0.26-0.43	0.40	0.20	0.26-0.54	0.05	0.24	14.29	0.42	0.35	0.02				
10 ³ ·(mm ³) ⁻¹		С	0.32	0.09	0.26-0.38	0.29	0.07	0.24-0.34	-0.03	0.11	-9.38	0.43	0.83	0.02				
GRA						Е	5.52	4.17	2.71-8.32	3.83	1.37	2.91-4.75	-1.69	4.39	-30.62	0.01	0.19	0.40
10 ³ ·(mm ³) ⁻¹	1.2-6.8	С	3.04	0.72	2.55-3.52	2.90	0.66	2.46-3.34	-0.14	0.98	-4.61	3.01	1.00	0.13				

Table 2. Changes in selected haematological (immunological) parameters (WBC, LYM, MON, GRA) after the ARDSV intervention.
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Data presented as mean \pm standard deviation and confidence intervals; *significant difference at p < 0.05 vs. the pre-intervention value; Δ and % difference with respect to the pre-intervention status; positive Δ indicates an increase in variables; p_p^2 – partial eta squared for repeated measurements; F – Fischer's F statistics for repeated measurements; p-value (post-hoc) – Scheffé's test for pairwise post-hoc comparisons; E – experimental group, C – control group; WBC – white blood cells, LYM – lymphocytes, MON – monocytes, GRA – granulocytes



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4. Discussion

On the basis of previous studies on the use of ARDSV, the training performed in the present experiment was expected to promote the development of blood volume [17] and physical capacity [14, 9, 15]. Changes in blood volume during the training process are strongly related to the development of physical capacity. Increased blood volume allows an increase in the end-diastolic volume of the heart cavities, which, in accordance with the Frank-Starling law, favours an increase in stroke volume [36]. This effect is considered the main factor for the development of VO₂max [37]. In a study by Zatoń et al. [17], an increase in plasma volume that resulted from moderate-intensity training with ARDSV was accompanied by gains in aerobic physical capacity. Unexpectedly, the results of the present investigation do not confirm the increase in the plasma volume and VO₂max as an effect of training with ARDSV. Group E showed no changes in HCT or HGB that could suggest an alteration in blood volume consistent with the methodology by Dill and Costill [38]. Although the Dill and Costill equation is mainly applied to assess changes in plasma volume that develop during a training process [42].

The differences in the effects of training with ARDSV that arise from the comparison of the results presented in this paper and those obtained by Zatoń et al. [17] may be due to the variable conditions of performing the training effort. The magnitude of adaptive changes in the blood depends on the processes of haemolysis, haemodilution [43], and erythropoiesis [44]. The intensity of the above-mentioned processes is influenced by the type of physical activity [43], training intensity [45], the duration of workouts [46], oxygen content in the breathing air [47], and ambient temperature [48] and humidity [49]. Of these factors, the study by Zatoń et al. [17] and the study described in this paper differed in the mode of activity (cycling vs. swimming), conditions of body cooling (air convection vs. water convection at 27°C), and time of activity (3 additional training sessions per week of 60 minutes each vs. activity similar to that prior to the experiment, i.e. 2 sessions per week of 50 minutes each).

It seems that the mode of physical activity should not influence the appearance of changes in blood morphological indicators obtained as a result of training with ARDSV. There may be differences in the severity of haemolysis between numerous modes of exercise, which may result in variations in haematological adaptation [50]. However, cycling and swimming are physical activities during which the haemolysis levels are similar [51] and lower when compared with running [50]. It is more likely that the effects of training with ARDSV may be determined by thermal conditions. During cycling training, the cooling factor is air movement, while during swimming, it is the convection of fluid with much better thermal conductivity [52]. Therefore, cycling exercise with ARDSV may have been a stimulus to induce heat stress. Danek et al. [12] stated that during breathing with ARDSV, heated air from a previous exhalation was partially inhaled. Heat stress exercise promotes blood plasma expansion, leading to a decrease in HGB and HCT [49], which was observed in the study by Zatoń et al. [17]. In turn, the lack of changes in HCT or HGB as a result of swim training with ARDSV may be due to a more efficient thermal energy loss in the aquatic environment, as described by Nielsen and Davies [53]. In such an environment, the application of ARDSV may have been an insufficient stimulus to provoke heat stress; thus, the adaptive changes reported in the studies referred to did not occur.

Another factor that may explain the differences in the development of adaptation to the efforts applied in ARDSV training observed between the study by Zatoń et al. [17] and the present study is the training volume. In the study by Zatoń et al. [17], the training program with ARDSV consisted of three additional training sessions per week, implemented simultaneously with the routine training regime. In our study, the number of training sessions in the experimental period (twice a week) did not differ from that in the pre-experimental period. Therefore, it appears that the mere manoeuvre of adding ARDSV to the routine aquatic activity was too weak a stimulus to provoke developmental changes.

The results presented in this paper indicate that regular swimming training was a stimulus provoking changes in parameters characterizing red blood cell structure. The MCHC parameter decreased and the MCH and MCV parameters increased. The obtained results demonstrate that the observed change was not an effect of ARDSV application, as it occurred in both groups (E and C). The information available in the literature implies that training load modifications in competitive swimming can affect changes in haematological parameters. Santhiago et al. [54] reported that HCT and MCV decreased after a period of intensive endurance training but increased after a period of high-intensity training. Similarly, Mujika et al. [55] showed that a period of intensified training favoured an increase in MCV, MCH, and MCHC values, but the opposite changes occurred during the tapering period. For comparison, in the present study, the intensity of training performed before the experiment (65–75% HRmax) did not differ from that applied during the experiment (HR achieved at 60% VO2max in the incremental test). The only factor that changed was the way of determining the training intensity, as described above. The same number of weekly training sessions were also applied during the experiment and before its commencement. Therefore, in light of the aforementioned papers [55, 54], it is difficult to explain the changes in MCH, MCV, and MCHC described in the present study.

Several limitations of the current study must be considered. Firstly, the researchers strongly support sports-specific capacity testing [56]. For instance, a swimming ergometer or swimming flume would have better replicated the swimming task and enhanced the validity of the incremental exercise test [57], even a "2-speed test" – an incremental exercise test used to determine the anaerobic threshold using blood lactate measures [58]. However, evidence shows that there are no differences in VO₂max between simulated swimming on an incline bench, tethered swimming, and bicycle ergometry, in which both land-and water-based testing is a valid method of assessing VO₂max in swimmers [59].

Secondly, previous studies on ARDSV in swimmers applied an 8-week training program [15]. The current study employed a 6-week intervention to determine if a shorter duration protocol could induce similar effects. It is possible that 8 weeks is the minimum timeframe required to fully promote the beneficial adaptations of ARDSV.

Thirdly, a relatively small, homogeneous sample of healthy young adults were examined in the study. It was not controlled whether the participants implemented any changes to their diet and other aspects that might impact blood parameters during the intervention period.

Finally, performance improvement (specific test on a standard distance) between groups was not measured, which should be taken into account in further studies.

Shei [60] paid attention to the fact that the knowledge about ARDSV is useful for athletes. Hence, future research appears necessary and it should involve elite swimmers or sedentary populations to further investigate ARDSV intervention, including exercise intensity, exercise frequency, and tube volume. The present study may serve as a theoretical and methodological grounding for future research to provide more conclusive data on the effects of ARDSV on haematological parameters among swimmers of various skill levels.

5. Conclusions

Adding ARDSV to short-term, moderate-intensity swim training does not improve physical capacity or develop blood parameters related to oxygen transport and the immunological system in physically active individuals. The lack of changes may be due to low heat stress during efforts in an aquatic environment. It is also possible that the moderateintensity training performed twice a week for 6 weeks was too weak of a stimulus to trigger adaptive changes. Swimming coaches and instructors should consider this when planning training programs for swimmers of various skill levels.

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